

A New Newt of the Genus *Cynops* (Caudata: Salamandridae) from Guangdong, China

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Abstract We describe a new species of the genus *Cynops* from northeastern Guangdong, China. This new species is distinguished from its congeners by a combination of morphological and molecular characters. In morphology, it is characterized by distinctive irregular bluish grey spots on the dorsum from head to tail; irregular, bright orange blotches on venter, chin, underside of axillae, limbs, cloaca; one bright orange stripe in the middle of venter; and ventral tail orange red. Analyses of mitochondrial DNA data indicate that this new species forms one highly diverged lineage within the Chinese group of *Cynops*.

Keywords Caudata, Salamandridae, *Cynops glaucus* sp. nov., newt, Guangdong, molecular analysis

1. Introduction

The salamandrid genus *Cynops*, commonly known as the fire-bellied newts, ranges from Yunnan in southwestern China to Japan in East Asia. *Cynops* along with other three genera, including *Pachytriton*, *Paramesotriton*, and *Laotriton*, comprise the modern Asian newts (Steinfartz *et al.*, 2007; Weisrock *et al.*, 2006; Zhang *et al.*, 2008). Currently, eight species of *Cynops* are recognized (AmphibiaWeb, 2012). Except for *C. pyrrhogaster* Boie and *C. ensicauda* Hallowell, which are restricted to some Japanese islands, the other six species occur in mainland China. Three highland species, *C. cyanurus* Liu, Hu and Yang, *C. chenggongensis* Kou and Xing, and *C. wolterstorffi* Boulenger, inhabit pools and lakes on the Yunnan-Guizhou Plateau. The other three species, *C. orientalis* David, *C. orphicus* Risch, and *C. fudingensis* Wu, Wang, Jiang and Hanken, occur in the hills and mountains of southeastern China (Fei *et al.*, 2006; Wu *et al.*, 2010b; Zhao and Hu, 1984).

Recent species discoveries, aided by molecular analyses

and more thorough fieldwork in remote montane regions of southern China, suggest that the true species richness of modern Asian newts is probably grossly underestimated (Gu *et al.*, 2012; Li *et al.*, 2008a, 2008b; Nishikawa *et al.*, 2011a, 2011b, 2012; Wu *et al.*, 2009, 2010a, 2010b; Zhao *et al.*, 2008). Guangdong is located along the coast of southeastern China. Its subtropical moist climate and mountainous terrain were little influenced by the Quaternary glacial oscillations. Complex mountain ranges with habitat stability are likely responsible for the retention of old gene lineages (Chen and Bi, 2007; Wu *et al.*, 2004).

During recent fieldwork in northeastern Guangdong in May 2011 and August 2012, several specimens of the genus *Cynops* were collected from Mt. Lianhua near the known range of *C. orphicus* (Figure 1). The new specimens had distinct phenotypes and could not be assigned confidently to any known species. Combined with the morphological and molecular analyses based on our and previous studies (Wu *et al.*, 2010b, 2010c), we suggest these specimens to be a new species, which is described herein.

2. Materials and Methods

2.1 Taxon sampling Ten specimens of the new species

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Received: 8 January 2013 Accepted: 12 April 2013

were collected from Wuhua County, Guangdong, China (Figure 1). Following euthanization, tissues were taken from adult specimens and were preserved in 95% ethanol. Voucher specimens were fixed in 10% buffered formalin, and later transferred to 70% ethanol. The holotype and paratypes are deposited in the Museum of the Kunming Institute of Zoology, Chinese Academy of Sciences.

In total, 29 samples were used for the reconstruction of maternal relationships, among which four individuals were the new species. All known congeneric species were included except for *C. chenggongensis* and *C. wolterstorffi* (Table 1), which have not been collected since the 1990s and for which tissue samples were not available. Six samples of five species including *Laotriton laoensis*, *Lissotriton vulgaris*, *Pachytriton granulatus*, *Paramesotriton deloustali*, and *Triturus cristatus* were chosen as outgroup taxa based on the studies of Wu *et al.* (2010b) and Zhang *et al.* (2008). Voucher specimens, and their localities and GenBank accession numbers are given in Table 1.

2.2 Morphological examinations Measurements were made by one person using dial calipers with a precision of 0.01 mm. Following Wu *et al.* (2010b), 12 linear measurements were taken: total length (TTL; distance between the tips of snout and tail), snout-vent length

(SVL; from snout to anterior edge of the vent), tail length (TAL; from the posterior edge of the vent to the tail tip), maximum tail depth (MTAD), head length (HEL; from the tip of snout to the posterior edge of parotoid gland), maximum head width (MHEW), interocular distance (IOD; from the anterior corner of each eye), distance from the anterior corner of the right eye to the right nostril (END), inter-nostril distance (IND), distance between the axilla and the groin along the left side of the body (AGD), average length of both forelimbs (AFL), and average length of both hind limbs (AHL).

2.3 DNA extraction and sequencing Total genomic DNA was extracted using the phenol-chloroform method (Sambrook *et al.*, 1989). A 1113 bp fragment of the NADH dehydrogenase subunit two (*ND2*) and its flanking tRNAs (tRNA^{Trp} and a partial tRNA^{Ala}) was amplified using primers KIZL4437 (Yuan *et al.*, 2011) and 5081R (Wu *et al.*, 2010c). Two internal primers ND2-38R (5'-TATTCAAYCCTAARTGTGCR-3') and 4416F (Wu *et al.*, 2010c) were applied for sequencing. Standard polymerase chain reactions (PCR) were performed in a final volume of 15 µl with the following procedures: initial denaturation at 94°C for 5 min, 35 amplification cycles at 94°C for 1 min, annealing for 1 min at 52°C, extension for 1 min at 72°C. Final extension at 72°C

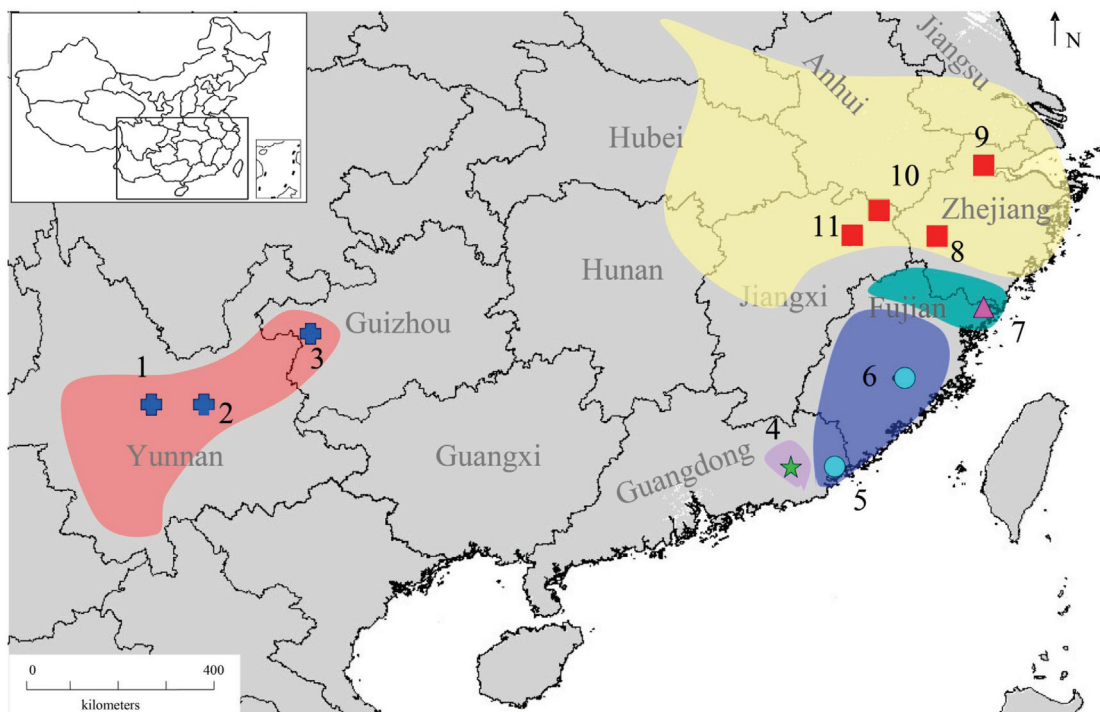


Figure 1 Map showing the distribution and sampling localities of *Cynops* in China used in this study. Five Chinese species include: *C. glaucus*, sp. nov. (green star), *C. orphicus* (sky-blue circles), *C. fudingensis* (pink triangle), *C. orientalis* (red squares), and *C. cyanurus* (blue diamonds). The range of each species is showed with shadow in different color based on the known localities documented from Fei *et al.* (2006) and Wu *et al.* (2010b). For locality numbers, see Table 1. Symbols correspond to lineages shown in Figure 2.

was conducted for 10 min. The successfully amplified products were purified using ExoSAP-IT purification kit according to the manufacturer's instruction. Purified PCR products were directly sequenced in both directions using a BigDye Terminator Cycle Sequencing Kit (version 2.0, Applied Biosystems, Foster City, California, USA) and an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems). Moreover, a 615 bp fragment of the gene encoding cytochrome oxidase subunit I (*COI*) for the new species was also amplified using the primers from Che *et al.* (2012). Due to the absence of *COI* sequences for other congeneric species, these newly obtained sequences of *COI* were not used for reconstructing the matrilineal genealogy.

2.4 Phylogenetic analysis Sequences were aligned using ClustalX 1.81 (Thompson *et al.*, 1997). Subsequently, the alignments were verified by eye. Genetic divergences

were estimated with MEGA 4.0 (Tamura *et al.*, 2007) by calculating the Kimura 2-parameter distance (K2P; Kimura, 1980).

We inferred maternal relationships of *Cynops* using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods. MP analyses were performed by PAUP* 4.0b10 (Swofford, 2003). Heuristic searches with tree-bisection-reconnection (TBR) were executed for 1000 random addition replicates with all characters treated as unordered and equally weighted. To assess nodal reliabilities, bootstrap analysis was conducted using 100 replicates. ML was performed using the program RAxML v.7.0.4 (Stamatakis *et al.*, 2008), with a GTR+I+G model used for all data with 100 bootstrap replicates. BI was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The data were partitioned based on codon position and tRNA. The best-fit evolutionary model was determined using the Akaike Information Criterion as implemented in MrModeltest

Table 1 Voucher specimens, localities and GenBank accession numbers for the species included in the molecular genealogical analysis. The numbers followed the localities correspond to site numbers in Figure 1. CIB: Chengdu Institute of Biology; GZNU: Guizhou Normal University; KIZ: Kunming Institute of Zoology; MVZ: Museum of Vertebrate Zoology (Berkeley, USA); SN: Not available. Specimen accession numbers marked as TP-MVZ are to be catalogued in the MVZ. *: Type locality; ND: ND2 and its flanking tRNAs.

Species	Voucher	Locality	GenBank No.		Source
			ND	COI	
Ingroup					
<i>Cynops cyanurus</i>	TP-MVZ 02	Chuxiong, Yunnan, China (1)	EU880309	No	Zhang <i>et al.</i> (2008)
	SN 02041	Kunming, Yunnan, China (2)	AY233157	No	Lu <i>et al.</i> (2004)
	SN 02042	Kunming, Yunnan, China (2)	AY233158	No	Lu <i>et al.</i> (2004)
	CIB 95897	Shuicheng, Guizhou, China (3) *	GU301784	No	Wu <i>et al.</i> (2010b)
	GZNU 20050727002	Shuicheng, Guizhou, China (3) *	FJ415604	No	Unpublished data
<i>C. glaucus</i> sp. nov. .	KIZ 09793	Wuhua, Guangdong, China (4)	KC762304	KC832685	This study
	KIZ 09799	Wuhua, Guangdong, China (4)	KC762306	KC832683	This study
	KIZ 09800	Wuhua, Guangdong, China (4)	KC762307	KC832684	This study
<i>C. orphicus</i>	MVZ 241427	Chao'an, Guangdong, China (5) *	DQ571772	No	Weisrock <i>et al.</i> (2006)
	MVZ 241428	Chao'an, Guangdong, China (5) *	EU880312	No	Zhang <i>et al.</i> (2008)
	MVZ 231158	Dehua, Fujian, China (6)	DQ517771	No	Weisrock <i>et al.</i> (2006)
<i>C. fudingensis</i>	CIB 97874	Fuding, Fujian, China (7) *	GU301785	No	Wu <i>et al.</i> (2010b)
	CIB 97875	Fuding, Fujian, China (7) *	GU301786	No	Wu <i>et al.</i> (2010b)
	CIB 97876	Fuding, Fujian, China (7) *	GU301787	No	Wu <i>et al.</i> (2010b)
<i>C. orientalis</i>	CIB 97919	Quzhou, Zhejiang, China (8) *	GU301790	No	Wu <i>et al.</i> (2010b)
	MVZ 230344	Hanzhou, Zhejiang, China (9)	DQ517770	No	Wu <i>et al.</i> (2010b)
	MVZ 230345	Hanzhou, Zhejiang, China (9)	EU880311	No	Zhang <i>et al.</i> (2008)
	CIB 88167	China	GU301783	No	Wu <i>et al.</i> (2010b)
	CIB 97864	Wuyuan, Jiangxi, China (10)	GU301789	No	Wu <i>et al.</i> (2010b)
	CIB 97867	Wannian, Jiangxi, China (11)	GU301788	No	Wu <i>et al.</i> (2010b)
<i>C. pyrrhogaster</i>	MVZ 263718	Chiba Prefecture, Japan	DQ517773	No	Weisrock <i>et al.</i> (2006)
<i>C. ensicauda</i>	MVZ 238580	Japan	DQ517769	No	Weisrock <i>et al.</i> (2006)
Outgroup					
<i>Laotriton laoensis</i>	FMNH 255452	Laos	EU880328	No	Zhang <i>et al.</i> (2008)
<i>Lissotriton vulgaris</i>	MVZ 230731	Vicinity of Tula City, Tula Oblast, Russia	EU880339	No	Zhang <i>et al.</i> (2008)
<i>Pachytriton granulosus</i>	CIB 88143	Mt. Tiantai, Ninghai, Zhejiang, China	GQ303610	No	Wu <i>et al.</i> (2010c)
<i>P. granulosus</i>	CIB 88145	Mt. Xitianmu, Lin'an, Zhejiang, China	GQ303606	No	Wu <i>et al.</i> (2010c)
<i>Paramesotriton deloustali</i>	MV Z223628	Vinh Yen District, Tam Dao, Vietnam	EU880327	No	Zhang <i>et al.</i> (2008)
<i>Triturus cristatus</i>	MV Z230726	Vicinity of Tula City, Tula Oblast, Russia	EU880336	No	Zhang <i>et al.</i> (2008)

v.2.3 (Posada and Crandall, 1998): TVM+I+G for the first codon position of *ND2*; K81uf+I+G for the second codon position of *ND2*; TrN+G for the third codon position of *ND2* and the tRNA region. Two independent runs were carried out for 5 000 000 generations. Trees were sampled every 100 generations. The first 25% of the trees were discarded as burn-in and log-likelihood scores were tracked to assure stabilization.

3. Results and Discussions

Eight sequences were newly determined and are deposited in GenBank (Table 1). Moreover, the four *COI* sequences are deposited to the Chinese Barcode of Life Database System. All the 29 aligned sequences of the *ND2* and its flanking tRNAs contain 1169 nucleotide sites, of which 553 were variable and 458 potentially parsimony-informative. No indels were observed, and no stop codons were found in protein coding regions, suggesting that nuclear pseudogenes were not sequenced.

The results yielded by MP, ML, and BI were largely congruent except for some nodes that received low support. Thus, only the BI tree is shown (Figure 2). The monophyly of *Cynops* receives low nodal support (Figure 2: BPP < 90), as previous studies have also shown

(Weisrock *et al.*, 2006; Zhang *et al.*, 2008). Among *Cynops*, all the Chinese newts form a monophyletic group with strong support (Figure 2: Chinese group, BPP = 100). The present tree depicts three distinct lineages (A, B, and C) within the Chinese group, although their relationships to one another are not resolved. Lineages A and B were supported by Wu *et al.* (2010b): one from southeastern China includes *C. orientalis*, *C. fudingensis*, and *C. orphicus* and the other from the Yunan Guizhou Plateau consists of *C. cyanurus*. Unexpectedly, compared with the data of Wu *et al.* (2010b), our data supports that newts from northeastern Guangdong near the type locality of *C. orphicus* (Figure 1) form a new distinct lineage C, which is firstly identified by present data. The genetic distance (K2P) between Lineage C and other congeneric species in the Chinese group ranges from 14.93% to 18.77% (Table 2). This level of genetic divergence is similar to that between *C. cyanurus* (17.29%–18.77%) and other congeners in the Chinese group, and higher than that between *C. fudingensis* (8.4%–17.29%), *C. orientalis* (8.4%–18.45%), *C. orphicus* (13.28%–17.94%) and other congeners in the Chinese group (Table 2). Compared with other salamandrid genera, such as *Lissotriton* (Babik *et al.*, 2005) and *Tylotriton* (Yuan *et al.*, 2011), the interspecific divergence in *Cynops* is distinctly higher.

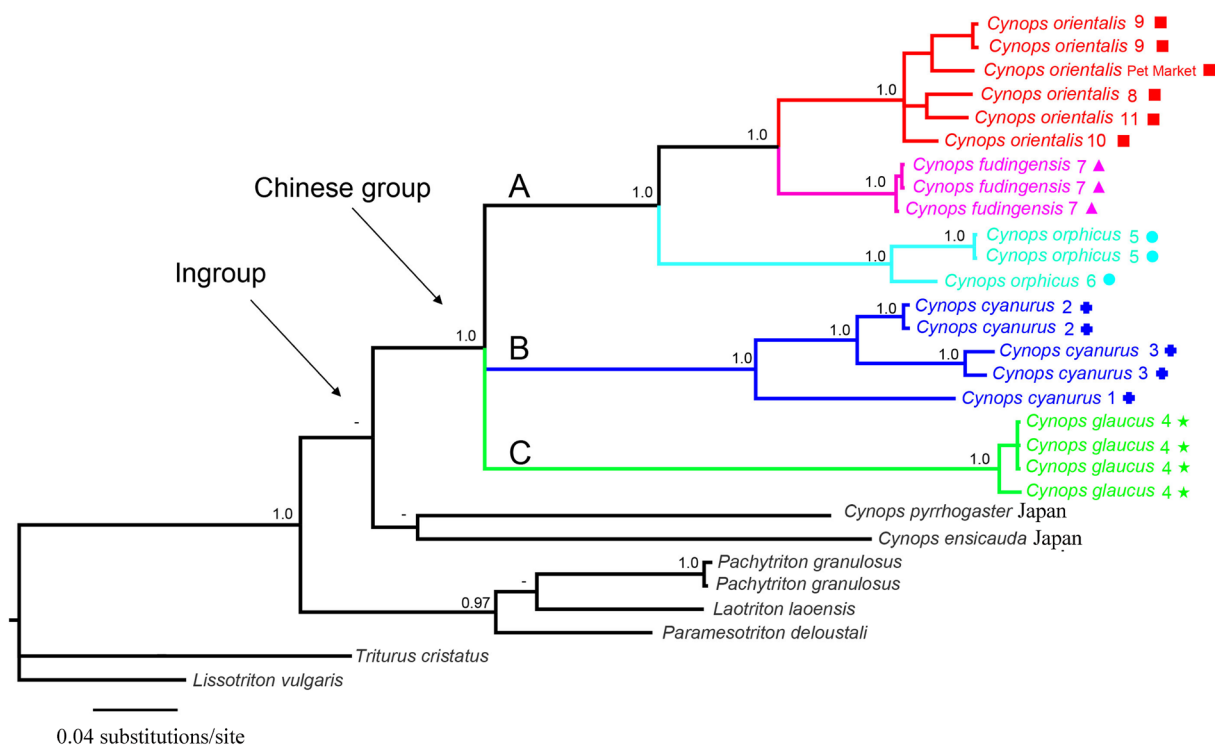


Figure 2 Bayesian inference (BI) tree based on *ND2* and flanking tRNA sequences. Numbers on branches are Bayesian posterior probabilities. - denotes low support (< 90%). Color of lineages, species name, and symbols correspond to Figure 1. For locality numbers, see Figure 1 and Table 1.

The matrilineal genealogy and genetic distances suggest that the new population from Wuhua County has been isolated for a long period of time and constitutes an undescribed species. This conclusion is supported by further time estimations (Yuan *et al.*, unpublished data) that Lineage C originated at about the middle Miocene (about 15 MYA). Future fine-scale sampling and historical analyses using a suite of additional genes, especially nuclear genes, will lead to a better understanding of the relationships and mechanisms of diversification among the species of *Cynops*.

4. Description of the New Species

Cynops glaucus sp. nov. (Figures 3, 4)

Holotype: KIZ 09791, an adult male from Meiguang Village (23.67° N, 115.80° E; elevation 742 m), in Mt. Lianhua, Wuhua County, Meizhou, Guangdong, China, collected by Zhiyong YUAN on 1 May 2011.

Paratypes: Seven adult males: KIZ 09790, KIZ 09792–09797, and two adult females: KIZ 09799–09800; same collection date and locality as the holotype.

Diagnosis: A small-sized (TTL less than 100 mm) species assigned to the genus *Cynops* based on the absence of a bony or warty ridge on head; skin finely granulated;

vertebral ridge present; and molecular phylogenetic evidence. The species differs from congeners by the following combination of characters: 1) postocular orange spot absent; 2) parotoid gland poorly developed; 3) vertebral ridge inconspicuous; 4) irregular bluish grey spots on dorsal sides of head, body, limbs and tail; 5) an irregular, bright orange stripe in the middle of venter through the neck to the front of the legs; 6) bright orange blotches on the chin, the venter and around cloaca; 7) bright orange blotches on underside of axillae and the four limbs; and 8) irregular black spots on tail.

Description of the holotype: The male holotype 35.51 mm SVL, 30.82 mm TAL (Measurements are given in Table 3); head oval in dorsal view; snout relatively blunt; projecting slightly beyond mandible; nostrils small, but conspicuous on snout tip; eye small, not extending beyond lateral margins of head; labial fold developed on posterior part of upper jaw; gular fold absent; an inconspicuous longitudinal ridge found posterior to each eye; tongue elongate, enlarged anteriorly, with free lateral margin; maxillary and mandibular tooth rows tiny and vomerine teeth arranged in “^” pattern; parotoid gland poorly developed, gill remnants absent; skin with fine granules, covering most parts of dorsal and ventral body; a few longitudinal wrinkles present on chin; vertebral

Table 2 K2P distances (%) of the mtDNA sequences for *ND2* and its flanking tRNA (tRNA^{Trp} and a partial tRNA^{Ala}) between the species of *Cynops*.

	<i>C. cyanurus</i>	<i>C. glaucus</i> sp. nov.	<i>C. orphicus</i>	<i>C. fudingensis</i>	<i>C. orientalis</i>	<i>C. pyrrhogaster</i>	<i>C. ensicauda</i>
<i>C. cyanurus</i>	-						
<i>C. glaucus</i> sp. nov.	0.1877						
<i>C. orphicus</i>	0.1769	0.1794					
<i>C. fudingensis</i>	0.1729	0.1493	0.1328				
<i>C. orientalis</i>	0.1845	0.1653	0.1384	0.0840			
<i>C. pyrrhogaster</i>	0.1833	0.1671	0.1649	0.1443	0.1586		
<i>C. ensicauda</i>	0.2093	0.2177	0.1969	0.1749	0.1798	0.1591	-

Table 3 Linear measurements (in mm) of *Cynops glaucus* sp. nov. Abbreviations are provided in the text. The holotype is included in the summary statistics for males.

Measurements	Holotype (KIZ 09791)	Males (n = 8)		Females (n = 2)	
		Range	Mean ± SE	Range	Mean ± SE
SVL	35.51	34.01–39.41	35.72 ± 0.64	42.59–48.84	45.72 ± 3.12
TTL	71.18	65.17–74.50	69.28 ± 1.23	83.40–95.93	89.67 ± 6.27
TAL	30.82	26.66–32.59	28.95 ± 0.74	36.11–42.59	39.35 ± 3.24
HEL	11.64	11.64–13.64	12.26 ± 0.25	13.14–15.53	14.35 ± 1.20
MHEW	8.76	8.10–9.75	8.77 ± 0.17	9.48–11.18	10.33 ± 0.85
END	2.50	2.31–2.90	2.52 ± 0.08	2.52–3.03	2.76 ± 0.26
IOD	4.98	4.64–5.32	4.90 ± 0.09	5.31–6.20	5.76 ± 0.45
IND	2.80	2.44–3.20	2.67 ± 0.09	2.56–3.24	2.90 ± 0.34
AGD	17.41	15.52–19.55	17.24 ± 0.41	21.41–23.65	22.53 ± 1.12
MTAD	5.18	5.12–6.34	5.67 ± 0.16	6.87–7.51	7.19 ± 0.32
AFL	12.62	12.24–14.73	13.20 ± 0.31	13.38–16.04	14.67 ± 0.97
AHL	13.06	12.61–15.01	13.69 ± 0.30	14.56–17.31	15.80 ± 1.23

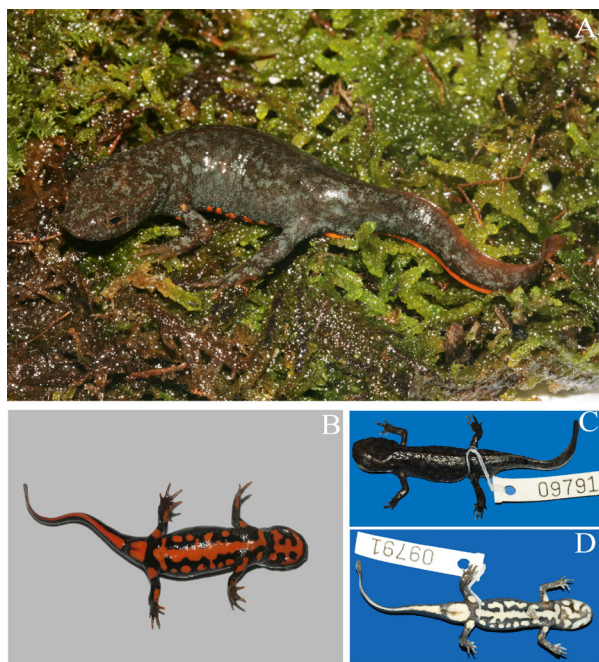


Figure 3 Photos of *Cynops glaucus* sp. nov. A: Dorsolateral view of living *C. glaucus* (KIZ 09800, paratype); B: Ventral view of living *C. glaucus* (KIZ 09800, paratype); C: Dorsal view of the holotype (KIZ 09791) in preservative; D: Ventral view of the holotype in preservative.

ridge elevated and inconspicuous; cloacal opening olive-shaped, slightly protruding, no papillae found on cloacal wall; four fingers and five toes slender, without webbing; relative length of fingers: $1 < 4 < 2 < 3$, and relative length of toes: $1 < 5 < 2 < 4 < 3$; tail slender, compressed laterally, tapering posteriorly; caudal fin distinct; and tail tip bluntly pointed.

Coloration of the holotype: Dorsum brownish black; bluish-grey spots irregularly arranged on dorsal sides of head, body, limbs, and tail; vertebral ridge dark orange;

four limbs and upside of tail light khaki; a single pale-orange dot on dorsal side of each forelimb base; bright orange blotches on underside of the body and limbs, and around cloaca; and two fresh-orange stripes, one on the middle of venter through neck to front of legs and the other on ventral tail.

In preservative, dorsum brownish black; vertebral ridge brown; irregular bluish-grey spots still found on dorsal sides of head, body, limbs, and tail; light khaki and all bright orange coloration fading to yellowish white.

Variation: Measurements are given in Table 3. Morphology of paratypes resembles that of holotype except that the cloaca is wider and more swollen in males than in females. Tail is proportionally shorter, and wider in males. Venter stripe and blotch color are usually reddish, but pale yellow is also present in some specimens. Number, shape, and position of ventral orange-red blotches vary among individuals.

Etymology: The specific epithet *glaucus* is an adjective after the general bluish grey coloration in life. Because of its coloration, we name this species as Bluish Grey Newt for its English name and *Huilan Rongyuan* for its Chinese name.

Habitat and distribution: *Cynops glaucus* sp. nov. was found in small, still-water wetlands on a hillside of Mt. Lianhua (Figure 4 A, B). The wetlands are covered with tall shrubs and grasses, and scattered with puddles. The puddles are shallow with water < 0.2 m in depth and narrow in width, except that three puddles are found 0.4–0.6 m deep and 1–1.5 m wide. The aquatic substrate is composed of soft earth and decomposing vegetation. However, there should be more locations or places where more individuals of this species could exist according to the local people's saying. Years of forest protection by the

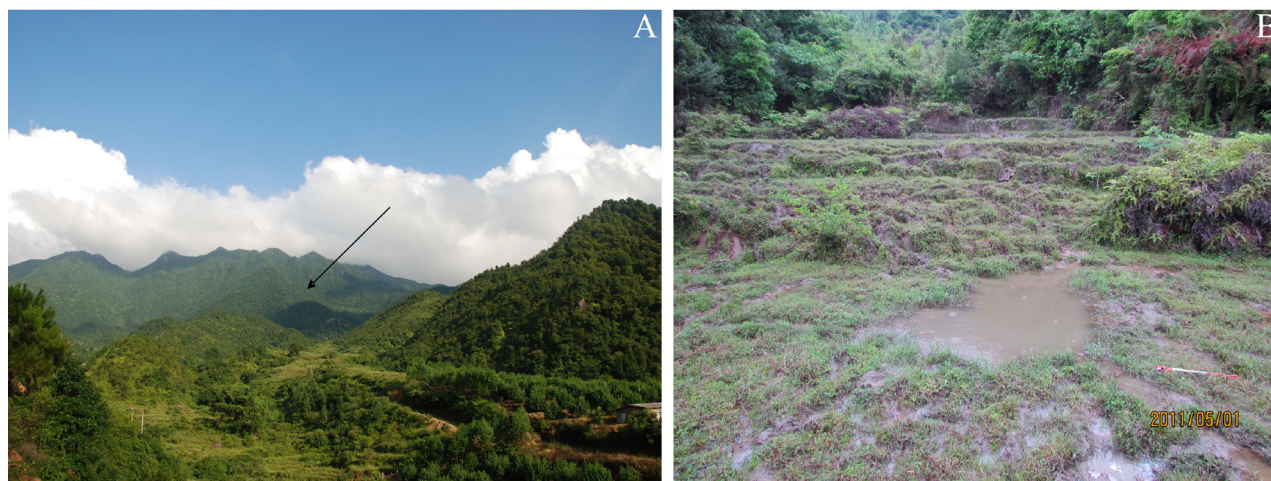


Figure 4 Type locality and habitat of *Cynops glaucus* sp. nov. A: Mt. Lianhua, Wuhua, Guangdong, China, the type locality of *C. glaucus*; B: The habitat of the holotype of *C. glaucus*. Arrow indicates the wetland in Mt. Lianhua where we collected the new species.

government resulted in dense overgrowth, thus attempts to find more localities failed during our two trips. Currently, *C. glaucus* sp. nov. is found only from the region near Mt. Lianhua in Wuhua County.

Comparison: *Cynops glaucus* sp. nov. is distinguished from other species of *Cynops* in having irregular bluish grey spots on dorsal sides of head, body, limbs and tail. Furthermore, it differs from *C. pyrrhogaster* and *C. ensicauda* by possessing inconspicuous vertebral ridge and having poorly developed parotoid gland. It differs from *C. cyanurus* and *C. chenggongensis* by absence of postocular orange spot. It differs from *C. orphicus* by having inconspicuous vertebral ridge. It differs from *C. fudingensis* by having these characters: an irregularly bright orange stripe in the middle of venter from neck to the front of legs; bright orange blotches on the chin, both left and right sides of the venter and around of cloaca; two bright orange blotches on the ventral sides of four limbs. It differs from *C. orientalis* by having a rough skin and possessing inconspicuous vertebral ridge.

Comparative material examined: *Cynops chenggongensis* (CIB 18434–39, from Yunnan, China), *C. cyanurus* (KIZ 011250–60, from Yunnan, China), *C. ensicauda* (KIZ 09757–58, from Ryukyu Islands, Japan), *C. orientalis* (KIZ 012940–013012; CIB 95564, CIB 95568–83, from Zhejiang, China), *C. fudingensis* (KIZ 012213–19; KIZ 09141–49, from Fujian, China); *C. orphicus* (KIZ 09838–51, from Fujian, China), *C. orphicus* (KIZ 09816–24, from Guangdong, China), and *C. pyrrhogaster* (KIZ 09755–56, from Kanagawa, Japan)

Acknowledgements We are grateful to the local administrative staff from Meiguang Village for their help in the field. We thank Weiwei ZHOU, Sang Ngoc NGUYEN and Hongman CHEN for photographing the specimens. Yuezhaoh WANG and Jiatang LI kindly assisted with examining specimens in CIB. We are grateful to Robert W. MURPHY, Theodore J. PAPENFUSS, Yang ZHONG and two anonymous reviewers for invaluable comments on the manuscript preparation. This work was supported by the grants from the Ministry of Science and Technology of China (MOST Grant 2011FY120200), the National Natural Science Foundation of China (31090250), the Chinese Academy of Sciences (KSCX2-YW-Z-0807, KSCX2-EW-Z-2, KSCX2-EW-Q-9), and the Bureau of Science and Technology of Yunnan, China (2010CI045).

References

- AmphibiaWeb:** Information on amphibian biology and conservation. 2012. Berkeley, California: AmphibiaWeb. Retrieved from <http://amphibiaweb.org/> (Accessed on 1 October 2012)
- Babik W., Branicki W., Cranobrnja-Isailovic J., Cogalniceanu D., Sas I., Olgun K., Poyarkov N. A., Garcia-Paris M., Arntzen W. 2005. Phylogeography of two European newt species – discordance between mtDNA and morphology. *Mol Ecol*, 14: 2475–2491
- Che J., Chen H. M., Yang J. X., Jin J. Q., Jiang K., Yuan Z. Y., Murphy R. W., Zhang Y. P. 2012. Universal COI primers for DNA barcoding amphibians. *Mol Ecol Res*, 12: 247–258
- Chen Y., Bi J. 2007. Biogeography and hotspots of amphibian species of China: Implications to reserve selection and conservation. *Curr Sci*, 92: 480–489
- Fei L., Hu S., Ye C., Huang Y. 2006. *Fauna Sinica, Amphibia Anura*, Vol. 1. Beijing, China: Science Press (In Chinese)
- Gu X. M., Chen R. R., Tian Y. Z., Li S., Ran J. C. 2012. A new species of *Paramesotriton* (Caudata: Salamandridae) from Guizhou Province, China. *Zootaxa*, 3510: 41–52
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*, 16: 111–120
- Li S., Tian Y., Gu X. 2008a. A new species of the genus *Paramesotriton* (Caudata, Salamandridae). *Acta Zool Sin*, 33: 410–413 (In Chinese)
- Li S., Tian Y., Gu X., Xiong R. 2008b. A new species of *Paramesotriton* — *Paramesotriton longliensis* (Caudata: Salamandridae). *Zool Res*, 29: 313–317 (In Chinese)
- Lu S. Q., Yuan Z. G., Pang J. F., Yang D. T., Yu F. L., McGuire P., Xie F., Zhang Y. P. 2004. Molecular phylogeny of the genus *Paramesotriton* (Caudata: Salamandridae). *Biochem Genet*, 42: 139–148
- Nishikawa K., Jiang J. P., Matsui M. 2011a. Two new species of *Pachytriton* from Anhui and Guangxi, China (Amphibia: Urodela: Salamandridae). *Curr Herpetol*, 30: 15–31
- Nishikawa K., Jiang J. P., Matsui M., Mo Y. M. 2011b. Unmasking “*Pachytriton labiatus*” (Amphibia: Urodela: Salamandridae), with description of a new species of *Pachytriton* from Guangxi, China. *Zoological Science*, 28: 453–461
- Nishikawa K., Matsui M., Jiang J. P. 2012. A new species of *Pachytriton* from China (Amphibia: Urodela: Salamandridae). *Curr Herpetol*, 31(1): 21–27
- Posada D., Crandall K. A. 1998. MODELTEST: Testing the model of DNA substitution. *Bioinformatics*, 14: 817–818
- Ronquist F., Huelsenbeck J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574
- Sambrook J., Fritsch E., Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd edition. New York: Cold Spring Harbor Laboratory Press
- Stamatakis A., Hoover P., Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol*, 57: 758–771
- Steinfartz S., Vicario S., Arntzen J. W., Caccone A. 2007. A Bayesian approach on molecules and behavior: Reconsidering phylogenetic and evolutionary patterns of the Salamandridae with emphasis on *Triturus* newts. *J Exp Zool Mol Dev Evol*, 308: 139–162
- Swofford D. L. 2003. PAUP*: Phylogenetic analysis using

- parsimony (and other methods), version 4.0b10. Sunderland, MA: Sinauer Associates
- Tamura K., Dudley J., Nei M., Kumar S.** 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software, version 4.0. *Mol Biol Evol*, 24: 1596–1599
- Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F., Higgins D. G.** 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl Acids Res*, 25: 4876–4882
- Weisrock D. W., Papenfuss T. J., Macey J. R., Litvinchuk S. N., Polymeni R., Ugurtas I. H., Zhao E. M., Jowkar H., Larson A.** 2006. A molecular assessment of phylogenetic relationships and lineage accumulation rates within the family Salamandridae (Amphibia, Caudata). *Mol Phylogenet Evol*, 41: 368–383
- Wu C. I., Shi S. H., Zhang Y. P.** 2004. A case for conservation. *Nature*, 428: 213–214
- Wu Y. K., Jiang K., Hanken J.** 2010a. A new species of newt of the genus *Paramesotriton* (Salamandridae) from southwestern Guangdong, China, with a new northern record of *P. longliensis* from western Hubei. *Zootaxa*, 2494: 45–58
- Wu Y. K., Rovito S. M., Papenfuss T. J., Hanken J.** 2009. A new species of the genus *Paramesotriton* (Caudata: Salamandridae) from Guangxi Zhuang Autonomous Region, southern China. *Zootaxa*, 2060: 59–68
- Wu Y. K., Wang Y., Jiang K., Hanken J.** 2010b. A new newt of the genus *Cynops* (Caudata: Salamandridae) from Fujian Province, southeastern China. *Zootaxa*, 2346: 42–52
- Wu Y. K., Wang Y., Jiang K., Hanken J.** 2010c. Homoplastic evolution of external colouration in Asian stout newts (*Pachytriton*) inferred from molecular phylogeny. *Zool Scr*, 39: 9–22
- Yuan Z. Y., Jiang K., Lu S. Q., Yang J. X., Nguyen Q. T., Nguyen T. T., Jin J. Q., Che J.** 2011. A phylogeny of the *Tylototriton asperrimus* group (Caudata: Salamandridae) based on a mitochondrial study: Suggestions for a taxonomic revision. *Zool Res*, 32: 577–584
- Zhang P., Papenfuss T. J., Wake M. H., Qu L., Wake D. B.** 2008. Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. *Mol Phylogenet Evol*, 49: 586–597
- Zhao E. M., Hu Q.** 1984. Studies on Chinese tailed amphibians. Chengdu, China: Sichuan Scientific and Technical Publishing House (In Chinese)
- Zhao H. T., Che J., Zhou W., Chen Y., Zhao H., Zhang Y. P.** 2008. A new species of *Paramesotriton* (Caudata: Salamandridae) from Guizhou Province, China. *Zootaxa*, 1775: 51–60